COSMO BIO CO., LTD.
Inspiration for Life Science

Mouse C-Peptide II EIA Kit

Cat. No. YII-YK012-EX

FOR LABORATORY USE ONLY

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Contents

– Please read all the package insert carefully before beginning the assay –

YK012 Mouse C - Peptide II EIA

. Introduction

This enzyme immunoassay (EIA) kit is a stable and convenient assay system for mouse C-peptide II in its plasma, serum and urine. The processing of proinsulin, which occurs within the B cell, yields insulin and C-peptide. The insulin and C-peptide are secreted in equimolar quantities into blood circulation. Therefore, the measurement of C-peptide in blood reflects the concentration of insulin and also provides valuable information to evaluate the pancreatic B cell function.

The EIA kit is prepared by using synthetic mouse C-Peptide II as standard and biotinylated mouse C-Peptide II as labeled antigen. The kit contains specific polyclonal antibody recognized to the amino acid sequence of mouse C-Peptide II.

We have already developed mouse C-peptide I EIA kit and mouse C-peptide I+II kit is also been developing in our laboratory.

YK012 Mouse C-Peptide II EIA Kit	Contents
▼ The assay kit can measure mouse C-Peptide II in the range of 0.412-100 ng/mL.	1) Antibody coated plate
▼ The assay completes within 16-18 + 2.5 hours.	2) C-Peptide II Standard
▼ With one assay kit, 41 samples can be measured in duplicate.	3) Labeled antigen 4) C-Peptide II antibody
▼ Test sample: mouse plasma, serum or urine Sample volume: 25 µL	5) SA-HRP solution
▼ The kit can be used in parts. Each raw (8 wells) of the microtiter plate can be detached and used separately.	6) Substrate buffer 7) OPD tablet
▼ Intra-assay CV(%) Serum 4.12-5.66 Inter-assay CV(%) Serum 2.72-10.62	8) Stopping solution 9) Buffer solution 10) Washing solution (concentrated)
▼ Stability and Storage Store all of the components at 2-8 . 6 months from the date of manufacturing. The expiry date is described on the label of kit.	11) Acetate plate sealer

. Characteristics

This ELISA kit is used for quantitative determination of mouse C-Peptide II in its plasma, serum & urine samples. The kit is characterized for sensitive quantification, high specificity and no influences with other components in samples. Mouse C-Peptide II standard is a highly purified synthetic product (purity: higher than 98%).

< Specificity >

The EIA kit shows following cross reactivity of 0.091% to mouse C-Peptide I, 0.34% to rat C-Peptide I, 63.3% to rat C-Peptide II and 1.8% to rat insulin and no cross reactivity to human and dog C-Peptide.

< Test Principle >

This EIA kit for determination of mouse C-Peptide II in serum, plasma and urine samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to mouse C-Peptide II and biotin-avidin affinity system. The 96 wells plate is coated with goat anti-rabbit IgG. C-Peptide II standard or samples, labeled antigen and rabbit anti mouse C-Peptide II antibody are added to the wells for competitive immunoreaction. After incubation and plate wash, HRP labeled streptoavidin (SA-HRP) are added to form HRP labeled streptoavidin-biotinylated mouse C-Peptide II-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of mouse C-Peptide II is calculated.

Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	MTP*1	1 plate (96 wells)	Goat anti rabbit IgG
2. C-Peptide II standard (100ng)	lyophilized	1 vial	Synthetic mouse C-Peptide II
3. Labeled antigen	lyophilized	1 vial	Biotinylated mouse C-Peptide II
4. C-Peptide II antibody	liquid	1 bottle (6 mL)	Rabbit anti mouse C-Peptide II
5. SA-HRP solution	liquid	1 bottle (12 mL)	HRP labeled streptoavidin
6. Substrate buffer	liquid	1 bottle (26 mL)	0.015% Hydrogen Peroxide
7. OPD tablet	tablet	2 tablets	o-Phenylenediamine dihydrochloride
8. Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
9. Buffer solution	liquid	1 bottle (25 mL)	Phosphate buffer
10. Washing solution (Concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
11. Acetate plate sealer		3 sheets	

MTP*1..... Microtiter plate

. Method

< Equipment required >

1) Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 492nm

2) Microtiter plate shaker

3) Washing device for microtiter plate and dispenser with aspiration system

4) Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips

5) Test tubes for preparation of standard solution

6) Graduated cylinder (1,000 mL)

7) Distilled water or deionized water

< Preparatory work >

1) Preparation of standard solution:
Reconstitute the C-Peptide II standard (lyophilized mouse C-Peptide II 100ng/vial) with 1mL of buffer solution, which affords 100ng/mL standard solution. The 0.1ml of the reconstituted standard solution is diluted with 0.2 mL of buffer solution that yields 33.33ng/mL standard solution. The 0.1mL of 33.33 ng/mL standard solution is diluted with 0.2 mL of the buffer solution, that makes 11.11ng/mL standard solution. Repeat the dilution to make each standard solution of 3.704, 1.235, and 0.412 ng/mL. Buffer solution is used as 0 ng/mL.

2) Preparation of labeled antigen:
Reconstitute labeled antigen with 12mL of buffer solution.

3) Preparation of substrate solution:
Resolve OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

4) Preparation of washing solution:
Dilute 50 mL of washing solution (concentrated) to 1000 mL with distilled or deionized water.

5) Other reagents are ready for use.

< Procedure >

1. Bring all the reagents to room temperature (20-30°C) before beginning the test.

2. Fill 25μL of each of standard solutions (0, 0.412, 1.235, 3.704, 11.11, 33.33, 100 ng/mL) or samples into wells first, then add 100μL of labeled antigen and finally introduce 50μL of C-Peptide II antibody into the wells.

3. Cover the plate with plate sealer and incubate it at 4°C for 16 - 18 hours. (Still, shaker not need)

4. After 4°C incubation, incubate it 1 hour at room temperature. During the incubation, the plate should be shake with a microtiter plate shaker.

5. Take off the plate sealer, aspirate the solution in the wells and wash the wells three times with approximately 0.35 mL/well of washing solution.

6. Pipette 100μL of SA-HRP solution into the wells.

7. Cover the plate with plate sealer and incubate it at room temperature for 1 hour. During the incubation, the plate should be shake with plate shaker.

8. Take off the plate sealer, aspirate and wash the wells five times with approximately 0.35 mL/well of washing solution.

9. Add 100μL of substrate solution into the wells cover the plate with plate sealer and incubate it for 30 minutes at room temperature.

10. Add 100μL of stopping solution into the wells to stop reaction.

11. Read the optical absorbance of the wells at 492nm. Calculate mean absorbance values of wells containing standards and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the standard curve to read C-Peptide II concentrations in samples from the corresponding absorbance values.

. Notes

1. Plasma, serum or urine samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30°C. Avoid repeated freezing and thawing of samples. EDTA-2Na additive blood collection tube is recommended for the plasma.

2. C-Peptide II standard, labeled antigen, substrate solution should be prepared immediately before use. Using clean test tubes or vessels in assay. Diluted washing solution is stable for 6 months at 2-8°C.

3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted.

4. Pipetting operations may affect precision of the assay, pipette standard solutions or samples precisely into each well of plate. In addition, use a new tip for each sample and standard to avoid cross contamination.

5. When sample value exceeds 100ng/mL, it needs to be diluted with buffer solution to a proper concentration.

6. During incubation except 4°C and color reaction, the test plate should be shake gently by microtiter plate shaker to promote immunoreaction.

7. Perform all the determination in duplicate.

8. The plate can be used for separately. In that case, reconstituted reagents (standard and labeled antigen) should be stored at or less than -30°C if be used within two weeks.

9. To quantitate accurately always run a standard curve when testing samples.

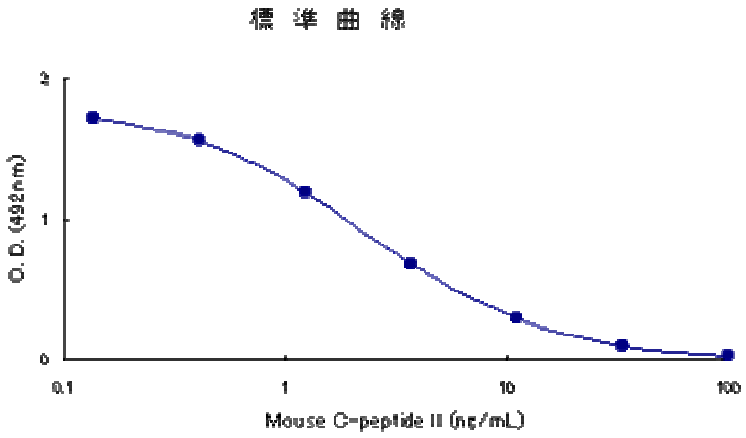
10. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

11. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

12. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

. Performance Characteristics

A typical standard curve



< Precision and reproducibility >

- Intra-assay CV(%) : Serum 4.12 ~ 5.66
- Inter-assay CV(%) : Serum 2.72 ~ 10.62

< Assay range >

0.412 – 100 ng/mL

< Analytical recovery >

Serum

Added (ng/mL)	Recovery (%)	SD （n=5)	CV (%)
0.520	107.624	4.846	4.503
1.042	110.917	4.044	3.646
2.083	108.311	3.990	3.684
4.167	120.291	4.683	3.893
8.333	116.770	8.377	7.174
16.667	114.817	5.646	4.918

Plasma

Added (ng/mL)	Recovery (%)	SD （n=5)	CV (%)
0.520	100.970	8.471	8.390
1.042	101.383	8.006	7.897
2.083	109.568	3.734	3.408
4.167	110.418	4.569	4.137
8.333	118.733	5.719	4.816
16.667	122.243	3.077	2.517

Urine

Added (ng/mL)	Recovery (%)	SD （n=4)	CV (%)
0.412	100.325	4.591	4.576
1.235	98.317	2.945	2.995
3.704	100.773	6.110	6.063
11.110	101.687	7.102	6.984
33.330	101.184	12.910	12.759

Remark:

The mouse urine was collected within 24 hours and diluted 10 folds with assay buffer before the test.

. Stability and Storage

< Storage >	Store all of the components at 2 to 8°C.
< Shelf life >	6 months from the date of manufacturing. The expiry date is described on the label of kit.
< Package >	For 96 tests per one kit including standards

. References

1. Wahren, J. et al: C-peptide makes a comeback. Diabetes Metab. Res. Rev., 19 (5), 345-347, 2003
2. Pierson, CR. et al: ProinsulinC-peptide replacement in type1diabetic BB/Wor-rats prevents deficits in nerve fiber regeneration. J. Neuropathol. Exp. Neurol., 62 (7), 765-79, 2003
3. Li, ZG. et al: C-peptide enhances insulin-mediated cell growth and protection against high glucose-induced apoptosis in SH-SY5Y cells. Diabetes Metab. Res. Rev., 19 (5), 375-85, 2003
4. Johansson, J. et al: Molecular efffects of proinsulin C-peptide. Biochem. Biophy. Res. Comm., 295, 1035-1040, 2002
5. Lindon, H. et al: C-peptide exerts cardioprotective effects in myocardical ischemia-reperfusion. Am. J. Phsiol. Heart Circ. Physiol., 279 (4), 1453-1459, 2000
6. Matsuda M. and Oka, Y: C-peptide (CPR), Nippon Rinsho Vol 57, 1999Suppl, Wide range blood・urine chemical detection. Immunological Examination (4) , 313-316, 1999
7. Wentworth, BM. et al: Characterization of the two nonallelic genes encoding mouse preproinsulin. J. Mol. Evol., 23 (4), 305-312, 1986
8. Yanaihara, C. et al: Immunoreactive rat C-peptide I and II in glucose perfusate of isolated pancreas. In: Insulin (ed. Brandenburg, D., and Wollmer. A.), Walter de Gruyter &Co., Berlin, 651-657, 1980
9. Yanaihara, C. et al: Immunological studies on synthetic rat and guinea pig C-peptide. In: Proinsulin, Insulin, C-peptide (ed.Baba,S., Kaneko,S., and Yanaihara, N.), Excerpta Medica, Amsterdam-Oxford, 87-93, 1979

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